

# Section I. (Amendments to the Claims)<sup>1</sup>

Please amend claims 1, 8, 12, 17-19, 27, 31, 71, 72 and 74 of the pending claims, as set out below.

The amendments of the claims are in the format proposed by the U.S. Patent and Trademark Office for adoption in July, 2003 revising 37 C.F.R. §1.121, pursuant to the Notice published on February 25, 2003 at 1267 OG 106, accepting current submissions of amendments in such revised format.

The claims pending in the application are claims 1, 3-10, 12-19, 21-28, 31-32, 62-72, 74-76 and 78-82.

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1. (currently amended) A fusion protein exhibiting a phase transition, the fusion protein comprising:

- (a) one or more biological molecules selected from the group consisting of peptides and proteins;

- (b) one or more phase transition proteins that exhibit an inverse phase transition, wherein the one or more phase transition proteins are joined to the biological molecule(s) of (a); and

- (c) optionally, a spacer sequence separating any of the phase transition protein(s) of (b) from any of the biological molecule(s) of (a),

wherein the fusion protein retains the inverse phase transition behavior of the phase transition proteins of (b) and wherein the insoluble phase of the fusion protein is of sufficient mass to be centrifugably removed from solution.

2. (previously cancelled)

3. (original) The fusion protein of claim 1 wherein the biological molecule of 1(a)

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<sup>1</sup> Consistent with the holding of *Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co., Ltd., et al.*, 535 U.S. 722, 152 L.Ed.2d 944 (2002), decided May 28, 2002, any amendments herein that hereafter are deemed to be narrowing amendments by a court of competent jurisdiction in a final unappealed or unappealable decision, are not intended to relinquish any scope of equivalents unforeseeable at the time of this amendment or that relate to aspects of the invention having only a peripheral relation to the basis for the amendment.

comprises a peptide.

4. (original) The fusion protein of claim 1 wherein the biological molecule of 1(a) comprises a biologically active protein.
5. (original) The fusion protein of claim 1 wherein the biological molecule of 1(a) comprises a therapeutic protein.
6. (original) The fusion protein of claim 1 wherein the biological molecule of 1(a) comprises an enzyme useful in industrial biocatalysis.
7. (original) The fusion protein of claim 1 wherein the biological molecule of 1(a) comprises an antibody or antibody fragment.
8. (currently amended) The fusion protein of claim 7 wherein the antibody or antibody fragment has complex forming affinity for an antigenic protein ~~biological molecule~~ of interest, and wherein upon binding to the ~~biological molecule~~ antigenic protein of interest, the fusion protein retains some or all of its phase transition character.
9. (previously amended) The fusion protein of claim 1 wherein the phase transition is mediated by one or more means selected from the group comprising:
  - (a) changing temperature;
  - (b) changing pH;
  - (c) addition of solutes and/or solvents,
  - (d) side-chain ionization or chemical modification; and
  - (e) changing pressure.
10. (original) The fusion protein of claim 1 wherein the phase transition is mediated by means comprising raising temperature.
11. (previously cancelled)
12. (currently amended) The fusion protein of claim 1 wherein the one or more phase transition protein(s) of 1(b) comprises oligomeric repeats of the pentapeptide Val-Pro-

Gly-X-Gly, wherein X is any natural or non-natural amino acid residue, and wherein X optionally varies among oligomeric repeats.

13. (original) The fusion protein of claim 12 wherein the X component(s) of the oligomeric repeats comprise(s) a naturally-occurring amino acid residue.
14. (original) The fusion protein of claim 12 wherein the X component(s) of the oligomeric repeats comprise(s) a non-naturally-occurring amino acid residue.
15. (original) The fusion protein of claim 12 wherein the X component(s) of the oligomeric repeats comprise(s) one or more amino acid residues selected from the group consisting of: alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine residues.
16. (original) The fusion protein of claim 12 wherein any two or more of the oligomeric repeats are separated by one or more amino acid residues which do not eliminate the phase transition characteristic of the fusion protein.
17. (currently amended) The fusion protein of claim 12 wherein the ratio of Val-Pro-Gly-X-Gly oligomeric repeats to other amino acid residues of the phase transition protein(s) of 1(b) ELP is greater than about 75%.
18. (currently amended) The fusion protein of claim 12 wherein the ratio of Val-Pro-Gly-X-Gly oligomeric repeats to other amino acid residues of the phase transition protein(s) of 1(b) ELP is greater than about 85%.
19. (currently amended) The fusion protein of claim 12 wherein the ratio of Val-Pro-Gly-X-Gly oligomeric repeats to other amino acid residues of the phase transition protein(s) of 1(b) ELP is greater than about 95%.
20. (previously cancelled)
21. (previously amended) The fusion protein of claim 1 wherein the spacer sequence comprises a proteolytic cleavage site.
22. (original) The fusion protein of claim 1 wherein the fusion protein further comprises a signal peptide.

23. (original) The fusion protein of claim 22 wherein the signal peptide is cleavable from the fusion protein by enzymatic cleavage.
24. (original) The fusion protein of claim 22 wherein the signal peptide directs secretion of the fusion protein from the cell.
25. (previously amended) The fusion protein of claim 1 wherein the fusion protein is recombinantly produced.
26. (previously amended) The fusion protein of claim 1 wherein any of the biological molecule(s) of 1(a), phase transition protein(s) of 1(b), or spacer sequence of 1(c) (when present) is recombinantly produced.
27. (currently amended) A fusion protein exhibiting a phase transition, the fusion protein comprising:

- (a) one or more ~~protein(s) of interest~~ biological molecules selected from the group consisting of peptides and proteins;
- (b) one or more phase transition protein(s) that exhibit an inverse phase transition, wherein the one or more phase transition protein(s) are joined at a C- and/or N-terminus of a protein' to the biological molecule(s) of (a); and
- (c) optionally, a spacer sequence separating any of the phase transition protein(s) of ~~(a)~~ (b) from any of the biological molecules of (a), and the phase transition protein(s) of (b)

wherein the fusion protein retains the inverse phase transition behavior of the phase transition proteins of (b), and wherein the phase transition temperature of the fusion protein is from about 35°C to about 60°C.

28. (original) The fusion protein of claim 27 wherein the phase transition is mediated by means comprising raising temperature.

Claims 29-30 (previously cancelled)

31. (currently amended) A fusion protein exhibiting a phase transition, the fusion protein comprising:

(a) ~~a protein of interest~~ one or more biological molecules selected from the group consisting of peptides and proteins;

(b) a one or more phase transition proteins that exhibit an inverse phase transition, wherein the one or more phase transition proteins are joined at a C- and/or N-terminus of the protein of interest to the biological molecule(s) of (a); and

(c) optionally, a spacer sequence separating any of the phase transition the protein(s) of (a) ~~(b)~~ from the ~~phase transition protein of (b)~~ any of the biological molecule(s) of (a),

wherein the fusion protein retains the inverse phase transition behavior of the phase transition proteins of (b), and with the proviso that when the biological molecule of (a) is glutathione S-transferase and the spacer sequence of (c) is proteolytically cleavable, the spacer sequence of (c) is cleavable by a protease agent selected from the group consisting of cysteine, aspartyl and metallo-proteases.

32. (original) The fusion protein of claim 31 wherein the phase transition is mediated by raising temperature.

Claims 33-61 (previously cancelled)

62. (withdrawn) A method of optimizing size of an ELP expression tag incorporated in a polynucleotide comprising a nucleotide sequence encoding a fusion protein exhibiting a phase transition, wherein the fusion protein comprises a protein of interest, said method comprising the steps of (i) forming a multiplicity of polynucleotides comprising a nucleotide sequence encoding a fusion protein exhibiting a phase transition, wherein each of said multiplicity of polynucleotides includes a different-sized ELP expression tag, (ii) expressing corresponding fusion proteins from said multiplicity of polynucleotides, (iii) determining a yield of the desired protein for each of said corresponding fusion proteins, (iv) determining size of particulates for each of said corresponding fusion proteins in solution as temperature is raised above  $T_0$ , and (v) selecting an optimized size ELP expression tag according to predetermined selection criteria for maximum recoverable protein of interest from among said multiplicity of polynucleotides.
63. (withdrawn) A method of purification of fusion proteins to yield a protein of interest, comprising forming a polynucleotide comprising a nucleotide sequence encoding a fusion protein exhibiting a phase transition, expressing the fusion protein in culture, and

subjecting a fusion protein-containing material from said culture to processing involving centrifugation and inverse transition cycling to recover said protein of interest.

64. (withdrawn) The method of claim 63, comprising expressing the fusion protein in culture in a well of a microplate.
65. (withdrawn) The method of claim 63, comprising processing the fusion protein-containing material from said culture in a well of a microplate.
66. (previously added) The fusion protein of claim 9, wherein the phase transition is mediated by addition of solute.
67. (previously added) The fusion protein of claim 66, wherein the solute comprises an organic solute.
68. (previously added) The fusion protein of claim 66, wherein the solute comprises an ionic solute.
69. (previously added) The fusion protein of claim 68, wherein the ionic solute comprises a salt.
70. (previously added) The fusion protein of claim 66, wherein the salt comprises NaCl.
71. (currently amended) An elastin-like peptide fusion protein comprising a protein of interest and an ELP component coupled by a cleavage site in a composition comprising a solvent medium in which the ELP fusion protein exhibits an inverse phase transition upon a predetermined change of composition condition wherein the phase transition is mediated by at least one change selected from the group consisting of:
  - (a) changing temperature;
  - (b) changing pH;
  - (c) addition of solutes and/or solvents;
  - (d) side-chain ionization or chemical modification; and

(e) changing pressure.

72. (currently amended) The ELP fusion protein of claim 71, ~~comprising wherein the a~~ protein of interest is cleavable from the ELP at a the cleavage site ~~of the ELP fusion protein~~ to yield the protein of interest and the ELP as cleavage products.
73. (previously cancelled)
74. (currently amended) The ELP fusion protein of claim ~~71~~ 72, wherein said composition further comprises a cleavage agent effective to cleave the cleavage site of the ELP fusion protein to yield the protein of interest and the ELP as cleavage products.
75. (previously added) The ELP fusion protein of claim 74, wherein said cleavage agent is a proteolytic agent effective to proteolytically cleave the cleavage site of the ELP fusion protein.
76. (previously added) The fusion protein of claim 12 wherein the phase transition protein(s) comprise a  $\beta$ -turn structure.
77. (currently cancelled)

*D/* [Please add new claims 78-85.]

78. (new) The fusion protein of claim 27 wherein the phase transition temperature is from about 38 to about 45°C.
79. (new) The fusion protein of claim 1 wherein the phase transition protein(s) of (b) are joined to the N-terminus of the biological molecule(s) of (a).
80. (new) The fusion protein of claim 21 wherein the proteolytic cleavage site is cleavable by a protease agent selected from the group consisting of cysteine, aspartyl and metallo-proteases.
81. (new) A fusion protein exhibiting a phase transition, the fusion protein comprising:
- (a) one or more biological molecules selected from the group consisting of peptides,

therapeutic proteins and antibodies or antibody fragments;

(b) one or more phase transition proteins that exhibit an inverse phase transition, wherein the one or more phase transition proteins are joined to the biological molecule(s) of (a); and

(c) optionally, a spacer sequence separating any of the phase transition protein(s) of (b) from any of the biological molecule(s) of (a),

wherein the fusion protein retains the inverse phase transition behavior of the phase transition proteins of (b).

82. (new) A fusion protein exhibiting a phase transition, the fusion protein comprising:

(a) one or more biological molecules selected from the group consisting of peptides, and proteins;

(b) one or more phase transition proteins that exhibit an inverse phase transition, wherein the one or more phase transition proteins are joined to the biological molecule(s) of (a); and

(c) optionally, a spacer sequence separating any of the phase transition protein(s) of (b) from any of the biological molecule(s) of (a),

wherein the fusion protein retains the inverse phase transition behavior of the phase transition proteins of (b), and wherein the spacer sequence of (c) is proteolytically cleavable by a protease agent selected from the group consisting of cysteine, aspartyl and metallo-proteases.

83. (new) A method of conducting biocatalysis including the steps of adding a biocatalytic enzyme to a solution to facilitate biocatalysis therein in production of a product, and isolating the enzyme from the solution to separate the enzyme from the product, wherein the enzyme comprises an enzyme-fusion protein (EFP), wherein the EFP comprises an ELP.

84. (new) The method of claim 83, wherein the enzyme after separation is recycled for subsequent rounds of biocatalysis.



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85. (new) A biocatalysis process, comprising use of an enzyme-fusion protein including an ELP to facilitate said biocatalysis.
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